PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/002556	10.03.2005	20.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68, C12N9/88, C12N15/52

Applicant
DEGUSSA AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/002556

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-11 (partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 1-11 (partially) insofar as inventions 2-28 are concerned.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/EP2005/002556

Re Item I
Basis of the report

1. This report relates to invention 1 as identified by the ISA.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

2. No opinion will be given on inventions 2-28, as they have not been covered by the ISR.

3. Although the subject-matter of claim 6 and its dependent claims is not part of this report, the following should be noted:

Present claim 6 relate to protein sequences defined by reference to desirable characteristics or properties, namely that the protein sequences would be able to construct the activity of nitrile hydratases and that their nucleic acids would give a positive hybridization signal with the primers of claim 1. The claims cover all the protein sequences having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such protein sequences. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT), as the aforementioned characteristic or properties are an attempt to define the protein sequences by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search (if requested) for claims 6-11 will be carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the protein sequences of SEQ. ID. Nos.: 38-86 (even-numbers) and to their nucleic acid sequences of SEQ. ID. Nos: 37-85 (odd-numbers).

Re Item IV

Lack of unity of invention

4. This Authority has found the following inventions:

Invention 1: Claims 1-5 (all partially): Degenerate primers of SEQ. ID. Nos. 1, 5 and 9 and processes for preparing nitrile hydratases using said degenerate primer.

Inventions 2-3: Claims 1-5 (all partially): Degenerate primers of claim 1 (except that of SEQ. ID. No.: 1), and processes for preparing nitrile hydratases using said degenerate primers (each primers defines a different invention) grouped as follows:

Invention 2: SEQ. ID. Nos: 2, 3, 6, 7, 10 and 11.

Invention 3: SEQ. ID. NOs: 4, 8 and 12.

Inventions 4-28: Claims 6-11 (all partially): Protein sequences of SEQ. ID. Nos.: 38-86 (even numbers), nucleic acids thereof of SEQ. ID. Nos: 37-85 (odd-numbers), nitrile hydratases constructed with said protein sequences, and expression systems and uses of said proteins and nucleic acids (Each protein sequence and its nucleic acid constitutes a different invention).

4.1. The present application does not comply with the requirements of unity of invention.

At least 28 separate inventions have been identified. Each of them is characterised by an individual "special technical feature"; there is no technical interrelation between these inventions (see below).

4.2. The following arguments reflect the preliminary opinion of the ISA concerning unity of invention:

4.2.1 Rule 13(2) PCT demands that "Rule 13.1 PCT shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression 'special technical features' shall

mean those technical features which define a contribution which each of the claimed invention considered as a whole makes over the prior art."

The PCT Preliminary Examination Guidelines C-III 7.6 state more precisely that "if the common matter of the independent claim is well known, and the remaining subject-matter ... differs without there being any unifying novel concept common to all of them, then clearly there is lack of unity".

4.2.2. The presently claimed subject-matter does not fulfil the necessary requirements on unity of invention as outlined above:

Claim 1 relates to the degenerate primers of SEQ. ID. Nos: 1-12.

Claims 2-5 relate to screening processes using the primers of claim 1 to isolate nitrile hydratases.

Claims 6-11 relate to nitrile hydratases, their nucleic acids and uses thereof.

4.2.3. In view of the disclosure of the present application, the technical problem to be solved is the provision of nitrile hydratases (p. 6, l. 8-11 of the application).

The degenerate primers of claim 1 should be considered as the alleged common technical feature between claims 1 and 2 and between claim 1 and 6, as said feature is mentioned on both claims 2 and 6.

Thus, claim 2 uses the primers of claim 1, which renders said claims unitary.

However, claims 1 and 6 cannot be considered unitary. Claim 6 is directed to compounds, protein sequences, which are encoded by nucleic acids identified by a hybridization process with the primers of claim 1.

As regards to the unity of the compounds of claims 1 and 6, the compounds of claim 1 do not encompass the same technical features as the compounds of claim 1, as required to acknowledge unity between products and intermediates of a process. Thus, the proteins of

claim 6 do not share any common structural element with the primers of claim 1, as amino acids and nucleotides are structurally different compounds.

As regards to the unity of the screening process of claim 2 or the process feature of claim 6 and the compounds identified by said process, namely, the protein sequences of claim 6, it should be mentioned that the present formulation of claim 6 is not allowable according to the PCT Guidelines 5.26 and 5.27, and it should be constructed as a product claim without reference to process features. Furthermore, screening methods, as those of present claim 2 or the process part of claim 6, and the compounds identified by said methods are not unitary according to the PCT Guidelines, 10.58, if the description is silent with regard to the relationship between the structure of the primer and the structure of the protein sequences. Even if the nucleic acids that encode the protein sequences of claim 6 and that hybridize with the primers of claim 1 would be considered as the relationship mentioned in the PCT Guidelines, 10.58, this feature could not be regarded as the special technical feature according to Rule 13.1 PCT as nucleic acids that hybridize with the primers of claim 1 are known in the art, as a sequence alignment would demonstrate. This is the case of the EMBL entries of ID Nos: PPU89363 or RRLNHASE, as they comprise the sequences GCCAAGGCCTGG and GCCGCGCCTGG, respectively. Moreover, the present screening processes of claim 2 or process part of claim 6 are not specially adapted to the manufacture of the present protein sequences as required by the Administrative instructions under the PCT, annex B, point (e), as those sequences disclaimed in claim 6 would also be obtained.

In view of the prior art and comments (supra), the technical content of the present application has to be rearranged into at least 2 individual objective problems with independent solutions:

Group 1: Claims 1-5: Provision of degenerate primers and their use in screening methods.

Group 2: Claims 6-11: Provision of nitrile hydratases.

4.2.4. As regards to the subject-matter of group 1 (claims 1-5), the available prior art discloses at least one solution to the said technical problem; moreover, the prior art solution shows the following technical features:

very limited number of such protein sequences. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT), as the aforementioned characteristic or properties are an attempt to define the protein sequences by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the protein sequences of SEQ. ID. Nos.: 38-86 (even-numbers) and to their nucleic acid sequences of SEQ. ID. Nos: 37-85 (odd-numbers).

The available prior art, namely the EMBL entries of ID Nos: PPU89363 (whole document) or RRLNHASE (whole document) or WO9712964 (Abstract and examples), discloses nitrile hydratases.

Therefore, the feature "nitrile hydratase" cannot be regard as the common technical link which could be the unifying concept between protein sequences of SEQ. ID. Nos: 38-86 (even-numbers).

Therefore further unified solutions should relate to groups of compounds sharing a common structural element that could be considered as the special technical feature providing unity: Thus, Rule 13.2 PCT should be considered to be met when the different inventions share a common chemical structure which constitutes a structurally distinctive portion in view of the existing prior art.

However, each sequence should be considered as a different technical feature that makes a contribution over the prior art disclosed in the EMBL entries of ID Nos: PPU89363 or RRLNHASE or WO9712964, since said sequences do not share any common chemical structure among them which constitutes a structurally distinctive portion in view of the existing prior art and therefore define different/alternative inventions.

Therefore, claims 6-11 encompass the aforementioned inventions 4-28.

4.3. As no other technical feature can be distinguished which, in the light of the prior art,

could be considered as the unifying concept, there is lack of unity between the plurality of claimed inventions defined in the application (Rule 13.1 PCT).

4.4 In conclusion, the groups of the aforementioned inventions are not linked by common or corresponding special technical features and define 12 different inventions not linked by a single general inventive concept.

The application, hence does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

5. Reference is made to the following documents:

D1: WO 97/12964 A

D2: PRECIGOU S ET AL: "Rapid and specific identification of nitrile hydratase (Nhase)-encoding genes in soil samples by polymerase chain reaction" FEMS MICROBIOLOGY LETTERS, AMSTERDAM, NL, vol. 204, no. 1, 2001, pages 155-161, XP002248648 ISSN: 0378-1097

D3: LORENZ PATRICK ET AL: "Screening for novel enzymes for biocatalytic processes: Accessing the metagenome as a resource of novel functional sequence space." CURRENT OPINION IN BIOTECHNOLOGY, vol. 13, no. 6, December 2002 (2002-12), pages 572-577, XP002330804 ISSN: 0958-1669

D4: LORENZ P ET AL: "Metagenome: A challenging source of enzyme discovery" JOURNAL OF MOLECULAR CATALYSIS. B, ENZYMATIC, ELSEVIER, AMSTERDAM,, NL, no. 19-20, 2 December 2002 (2002-12-02), pages 13-19, XP002319921 ISSN: 1381-1177

D5: DATABASE EMBL [Online] 12 February 1992 (1992-02-12), "R.rhodochrous gene for L-NHase" XP002331764 retrieved from EBI accession no. EM_PRO:RRLNHASE Database accession no. RRLNHASE

D6: DATABASE EMBL [Online] 19 March 1997 (1997-03-19), "Pseudomonas putida P38K, amidase, nitrile hydratase alpha subunit, nitrile hydratase beta subunit,

and P14K genes, complete cds." XP002331765 retrieved from EBI accession no. EM_PRO:PPU89363 Database accession no. PPU89363

D7: LOPES LOURENCO PEDRO MIGUEL ET AL: "Searching for nitrile hydratase using the consensus-degenerate hybrid oligonucleotide primers strategy" JOURNAL OF BASIC MICROBIOLOGY, vol. 44, no. 3, 2004, pages 203-214, XP009048598 ISSN: 0233-111X

D8: LIEBETON K ET AL: "Identification and expression in E. coli of novel nitrile hydratases from the metagenome" ENGINEERING IN LIFE SCIENCES 2004 GERMANY, vol. 4, no. 6, 2004, pages 557-562, XP009048590 ISSN: 1618-0240

6. Novelty of invention 1:

The present primers of SEQ. ID: Nos: 1, 5 and 9 are novel. Therefore, the compounds claim 1 and the process claims 2-5 are novel for invention 1.

7. Inventive step of invention 1:

Nitrile hydratases and processes for the preparation of nitrile hydratases using metagenome DNA libraries and degenerate fragments are known from D2, which is considered the closest prior art.

The different with the present application is the use of the specific degenerate primers of SEQ. ID. NOs.: 1, 5 and 9.

The technical problem could be regarded as the provision of alternative degenerate primers.

The solution proposed is based on the provision of the primers of SEQ. ID. Nos: 1, 5 and 9 which are used in screening methods that result in the isolation of nitrile hydratases.

The solution proposed is not inventive, since the provision of further degenerate primers to those of the prior art, D1 and D2, is obvious to the skilled person, as they are based on conserved amino acid sequence motifs of nitrile hydratases. As novel nitrile hydratases are

discovered, novel motifs could be found by using routinary sequence comparison programs (see for instance, Fig. 2 of D4). Furthermore, an inventive contribution of this sequences to the methods of claim 2 is also not found. Therefore, the SEQ. ID. Nos.: 1, 5 and 9 are not inventive either by itself or by their contribution to the processes of claims 2.

The process of claim 2 cannot be considered inventive because of the use of an inventive primer, as said primers are not inventive. Furthermore, processes for the provision of further nitrile hydratases are obvious for the skilled person from the screening methods of D2, or from the combination of the screening methods of D2 with the degenerate primers of D1 or D4 (Fig. 2), or even from the combination of the screening methods of D1 and the general knowledge of D3 or D4, which teaches the use of metagenome DNA libraries as a powerful tool for isolating genes.

8. Industrial applicability:

The subject-matter of invention 1 is industrially applicable.

Re Item VII
Certain defects in the international application

9. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1, D3-D4 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

10. It would be desirable to improve the clarity of present claim 1 by introducing the meaning of the non-common bases mentioned as found in the Supplement 2 to the EPO OJ 11/1998.

Although the subject-matter of claim 6 and its dependent claims is not part of this report, the following should be noted:

11. Claim 6 is not supported by the description as required by Article 6 PCT, as its scope is broader than justified by the description, as said claim is directed to any nitrile hydratase with less than 100% homology to the known ones and which is encoded by a nucleic acid sequence which hybridizes with the primers of claim 1. Although the description shows some nitrile hydratases, it does not show that they hydridize with the primers of claim 1.

12. Present claim 6 relate to protein sequences defined by reference to desirable characteristics or properties, namely that the protein sequences would be able to construct the activity of nitrile hydratases and that their nucleic acids would give a positive hybridization signal with the primers of claim 1. The claims cover all the protein sequences having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such protein sequences. Therefore, the claims lack support, and the application lacks disclosure.

13. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). As the aforementioned characteristic or properties are an attempt to define the protein sequences by reference to a result to be achieved.